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Review

CSF-1 as a Regulator of Macrophage Activation and Immune Responses

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Abstract. Macrophage activation is a key determinant of susceptibility and pathology in a variety of inflammatory diseases. The extent of macrophage activation is tightly regulated by a number of pro-inflammatory cytokines (e.g. IFN-γ, IL-2, GM-CSF, IL-3) and anti-inflammatory cytokines (e.g. IL-4, IL-10, TGF-β). Macrophage colony-stimulating factor (CSF-1/M-CSF) is a key differentiation, growth and survival factor for monocytes/macrophages and osteoclasts. The role of this factor in regulating macrophage activation is often overlooked. This review will summarize our current understanding of the effects of CSF-1 on the activation state of mature macrophages and its role in regulating immune responses.

Key words: CSF-1; macrophage; lipopolysaccharide; Toll-like receptors; inflammation.

Introduction

The production of circulating monocytes and tissue macrophages from the bone marrow is dependent on colony-stimulating factor 1 (CSF-1). This is highlighted by the gross deficiencies in macrophage development and numbers that occur in the *op/op* mouse, which has a natural inactivating mutation in the CSF-1 gene (for review see[84]), and by the demonstration that administration of CSF-1 to mice caused a 10-fold increase in blood monocyte numbers and increased macrophage numbers in the liver, spleen and peritoneal cavity[41].

Via alternative splicing, post-translational modifications and proteolytic processing, CSF-1 is produced in multiple forms; a secreted homodimeric glycoprotein, a secreted proteoglycan and a membrane-bound glycoprotein[73, 84]. The CSF-1 receptor (CSF-1R), encoded by the *c-fms* protooncogene, is a type III receptor tyrosine kinase with structural similarity to c-kit and the platelet-derived growth factor receptor and the fms-like receptors flk, and flt 1, 2 and 3. Mice with a targeted disruption of the *c-fms* gene are essentially a phenocopy of the *op/op* mouse, indicating that all of the actions of CSF-1 are mediated by the CSF-1R[18]. Ligand binding to the CSF-1R initiates receptor dimerization, auto-

Abbreviations used: BMM – bone marrow-derived macrophage, CpG DNA – bacterial CpG-containing DNA, CSF-1 – colony-stimulating factor 1, CSF-1R – CSF-1 receptor, EBV – Epstein-Barr virus, HIV-1 – human immunodeficiency virus type 1, IDO – indoleamine 2,3-dioxygenase, IL – interleukin, LPS – lipopolysaccharide, MMP – matrix metalloproteinase, RA – rheumatoid arthritis, TLR – Toll-like receptor, TNF – tumor necrosis factor.

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phosphorylation, and activation of multiple signalling cascades including the mitogen-activated protein kinase and P-I-3 kinase/Akt pathways[21, 35].

Although relatively few studies have addressed the biological roles of the different CSF-1 isoforms, it is likely that they have distinct functions *in vivo*. For example, the proteoglycan form is localized to specific types of extracellular matrix which might enable specialized functions such as regulation of bone homeostasis[68]. Daily administration of soluble recombinant CSF-1 to the *op/op* mouse from day 3 of life was able to correct some but not all of the deficiencies in macrophage numbers in different tissues[13]. This supports the argument that the full biological activity of CSF-1 requires all isoforms and not just soluble circulating CSF-1, although it is also possible that pre-natal absence of CSF-1 has effects that cannot be subsequently restored by exogenous CSF-1. The gene regulatory elements that are sufficient to reconstitute normal CSF-1 expression *in vivo* have been defined[77]. Transgenic expression on the *op/op* background of cDNAs encoding the secreted, proteoglycan or membrane-bound isoforms under the control of these elements should therefore provide clear evidence for any isoform-specific functions.

Regardless of the functions of the different isoforms, it is clear that CSF-1 is abundantly present *in vivo*. The major source of circulating CSF-1 is thought to be endothelial cells that line blood vessels[76], but a range of other cell types, including fibroblasts, osteoblasts, monocytes, B cells, T cells and bone marrow stromal cells also produce CSF-1. In mice, CSF-1 levels are in the order of tens of nanograms per millilitre in the circulation and tens of picograms per milligram in a variety of tissues including liver, lung, spleen, kidney, intestine and heart[75]. CSF-1 levels are dramatically increased upon challenge with lipopolysaccharide (LPS)[75] or with infectious agents such as *Listeria monocytogenes*[15, 32] and *Candida albicans*[14]. In humans, a similar situation is apparent; CSF-1 levels were enhanced in patients with sepsis[29], and LPS administration to cancer patients increased CSF-1 levels[23]. These observations would suggest that CSF-1 is likely to regulate immune responses to infection in both mice and humans.

Macrophage-Priming by CSF-1

The requirement for the CSF-1/CSF-1R system in macrophage development makes interpretation of *in vivo* studies aimed at addressing the role of CSF-1 in immunological responses difficult, since modulation of CSF-1 action affects both monocyte/macrophage production and survival, as well as mature macrophage functions. Additionally, CSF-1 is required for the maintenance of protein synthesis. JESSUP et al.[43] showed that CSF-1 greatly increased acetylated low-density lipoprotein uptake and foam cell formation, but this was entirely attributable to an increase in cell size; no effect was apparent when these parameters were assessed on a per microgram protein basis. This absolute requirement on CSF-1 for normal macrophage function raises an important issue with regard to the many *in vitro* studies that are performed on this cell type. Since it is normally present *in vivo* and because its absence has gross effects on normal cellular functions, CSF-1 should be present in culture medium during *in vitro* studies on macrophage function. This is often not the case, particularly in immunological studies.

A role for CSF-1 in regulating macrophage activation and inflammation is supported by *in vitro* studies in which CSF-1 enhanced macrophage phagocytic activity against *Listeria monocytogenes*[16], microbicidal action against *Leishmania mexicana*[40], *Candida albicans*[47] and *Aspergillus fumigatus*[74] and primed tumoricidal activity upon triggering with activating agents[66, 79, 80]. In other studies, CSF-1 did not enhance the ability of macrophages to kill intracellular organsims, including *Leishmania major*[6], *Mycobacterium tuberculosis*[19] and *Listeria monocytogenes*[20]. The basis for this inconsistency is unclear.

In contrast to *in vitro* studies, *in vivo* studies strongly support a role for CSF-1 in regulating primary immune responses to infection. For example, mice challenged with *Listeria monocytogenes* had an increased bacterial load following administration of an anti-CSF-1 antibody at the time of challenge[32] and CSF-1 transgenic mice were more resistant to *Listeria monocytogenes* than wild-type controls[8]. CSF-1 administration also had protective effects for the host against challenge with *Listeria monocytogenes*[49] and *Candida albicans*[14].

As well as enhancing the ability of macrophages to destroy invading pathogens directly, CSF-1 is also a potent regulator of monokine production that regulates the magnitude of the inflammatory response. CSF-1 alone triggered production of mRNAs for interleukin (IL)-6, granulocyte macrophage (GM)-CSF, IL-1α and IL-1β in murine resident peritoneal macrophages[25, 45]. In other studies with different macrophage populations, CSF-1 alone did not trigger pro-inflammatory cytokine production, but instead acted as a potent priming signal for a subsequent activation stimulus. Pre-treatment of thioglycollate-elicited peritoneal macrophages or bone

marrow-derived macrophages (BMM) with CSF-1-enhanced production of key pro-inflammatory cytokines, including IL-6, IL-12 and tumor necrosis factor (TNF)-α, upon triggering with LPS[26, 45, 46, 86], TNF-α[26] or extracellular matrix proteins[51, 52]. Similar priming effects of CSF-1 have been reported for human monocytes[3, 80, 98]. These *in vitro* studies correlate with *in vivo* studies in which the *op/op* mouse was resistant to the toxic effects of LPS[87, 100], although the interpretation of this data is somewhat compromized by the defects in macrophage development in the *op/op* mouse.

Interestingly, the ability of CSF-1 to prime macrophage activation is dependent upon the nature of the activating stimulus. Whereas CSF-1 synergized with LPS for pro-inflammatory cytokine production from BMM, it suppressed these responses when the activating signal was bacterial CpG-containing DNA (CpG DNA) and had no effect when the stimulus was bacterial lipopeptide[86]. Hence, the role of CSF-1 may depend upon the type of infectious challenge. In Gram--negative infections where LPS will be detected, CSF-1 may act as a potent priming signal for macrophage activation, whilst in a Gram-positive infection CSF-1 may not be as critical in regulating macrophage function.

The differential effects of CSF-1 on responses to different activating signals is, in part, mediated by selective effects of CSF-1 on expression of Toll-like receptors (TLRs)[86], which are required for signaling in response to microbial products[89]. Whereas CSF-1 did not regulate expression of a variety of TLR members, including TLR3, 4, 5 and 7, expression of TLR9, which is required for responses to CpG DNA, was down-regulated by CSF-1. Levels of TLR2 and 6 mRNA, encoding receptors for Gram-positive components, were also moderately suppressed by CSF-1 (unpublished data and [86]).

The biological significance of the potent inhibitory effect of CSF-1 on TLR9 expression and responses to CpG DNA is not clear, but others have also reported that CSF-1 can in some cases inhibit macrophage activation. For example, CSF-1 pretreatment inhibited the macrophage respiratory burst when zymosan, a TLR2/ /TLR6 agonist[69], was used as the triggering signal[70]. Hence, CSF-1 may have dual roles in responses to infection; as a positive regulator of macrophage activation when extreme danger signals such as LPS are detected and to prevent excessive macrophage activation when less hazardous products such as CpG DNA and zymosan are encountered.

Mechanisms of CSF-1-Mediated Priming of Cytokine Production

In human monocytes/macrophages, the mechanisms by which CSF-1 primes activation have not been extensively studied. CSF-1 marginally increased expression of CD14 on human monocytes and this may partially account for increased sensitivity to LPS[3]. Whether CSF-1 regulates LPS recognition in murine macrophages has not been adequately addressed, but CSF-1 did not regulate expression of the receptor for LPS, TLR4 or early signaling events in response to maximal LPS doses[86]. Instead, CSF-1 appears to prime murine macrophage responses to LPS through a number of distinct mechanisms. The synergism between CSF-1 and either LPS or TNF-α for IL-6 production, from resident peritoneal macrophages was largely-mediated by CSF--1-stimulated autocrine GM-CSF production, since synergism was greatly reduced, although not abolished, in GM-CSF-deficient mice[26]. The mechanism by which CSF-1 augments IL-12 production is not known, but may also involve autocrine GM-CSF, since this factor synergises with LPS for IL-12 production (unpublished data and [39]). Another possibility is that synergy occurs at the transcriptional level, since Ets-2, which binds to and activates the IL-12 p40 promoter[56, 57], is activated in a sustained fashion in response to CSF-1[28]. The priming effect for TNF-α production is downstream of mRNA regulation since CSF-1 enhanced LPS-induced TNF-α secretion but not LPS-induced TNF-α mRNA levels in BMM[86]. Since CSF-1 is known to increase production of matrix metalloproteinase (MMP) 9[92], one possibility is that CSF-1 also induces production of the MMP-related protein TNF-α converting enzyme that is required for cleavage of membrane-bound TNF-α[60, 61].

CSF-1 as a Regulator of Leukocyte Recruitment

Apart from affecting macrophage activation, CSF-1 acts at multiple levels to regulate cellular recruitment, a hallmark of inflammatory responses. Firstly, CSF-1 has pronounced effects on macrophage motility. Treatment of the CSF-1-dependent murine macrophage cell line BAC1.2F5 with CSF-1 rapidly triggered actin reorganization, membrane ruffling and cell spreading[2, 11]. Further, CSF-1 had chemokinetic and chemotactic activity on human monocytes[96], BAC1.2F5 cells[99] and myeloid progenitor cells-transfected with a *c-fms* expression plasmid[71]. Thus, the enhanced production of CSF-1 during responses to infection may serve to re-

cruit monocytes rapidly to the inflammatory site. Secondly, CSF-1 regulates expression of many genes that encode mediators of adhesion and migration. These include the integrins, $\alpha 4\beta 1$ and $\alpha 5\beta 1$[82], urokinase plasminogen activator[36, 83], plasminogen activator inhibitors[17, 36, 101] and MMP9[92]. Finally, CSF-1 may enhance chemokine production from monocytes/macrophages, thereby allowing for recruitment of other effector cells; CSF-1 increased IL-8 production from human monocytes[38, 90]. The importance of CSF-1 for the recruitment of monocytes during inflammatory responses has also been documented *in vivo*. Blockade of CSF-1 action suppressed monocyte recruitment during *Listeria monocytogenes* infection[31] and renal inflammation[54].

CSF-1 Involvement in Viral Infections

Many viruses, including respiratory syncytial virus, measles virus, dengue virus and HIV-1, replicate within macrophages as a means of escaping immune detection, and in many cases CSF-1 regulates macrophage function during viral infection. This is particularly true for HIV-1, where CSF-1 is an important factor in the HIV-1 replicative strategy. Firstly, HIV-1 replication has been associated with increased CSF-1 production *in vivo*[30] and replication of HIV-1 within monocyte-derived macrophages triggered CSF-1 production[33, 53]. Secondly, CSF-1 enhanced HIV-1 replication in macrophages[7, 44], possibly by regulating expression of the HIV-1 co-receptor CCR5[97]. Thus, HIV-1 appears specifically to induce CSF-1 production from macrophages and this, in turn, enhances its ability to replicate within this cell type. CSF-1/CSF-1 receptor antagonists may therefore have therapeutic potential as agents to block HIV-1 replication within macrophages.

Whilst CSF-1 is a positive regulator of HIV-1 replication, it may be protective to the host for other viral pathogens. Intranasal administration of CSF-1 offered almost complete, protection to BALB/c mice upon challenge with otherwise lethal doses of Sendai virus[59], and CSF-1 treatment of macrophages *in vitro* induced resistance to vesicular stomatitis virus[55]. Interestingly, Epstein-Barr virus (EBV) encodes a protein, BARF1, that bound to and neutralized CSF-1[85]. Although the biological consequences of this have not been addressed, one might predict that blockade of CSF-1 signaling via BARF1 would provide a selective advantage to EBV over the host. Indeed, there are reports of CSF-1R expression on B cells[4, 91], which may be relevant to EBV infection. Whilst some of the anti-viral actions of CSF-1 may be attributed to production of type 1 and type 2 interferons[55, 59], one report demonstrated that a CSF-1 expression construct, when administered with a DNA vaccine, enhanced cytotoxic T lymphocyte responses[50]. Therefore, CSF-1 may also enhance the development of antigen-specific anti-viral mechanisms.

Effects of CSF-1 on the Acquired Response

Whilst most of the studies described above indicate that CSF-1 enhances macrophage activation and the innate response, CSF-1 is actually immunosuppressive for antigen-specific responses. T cell responses against allogeneic cells were suppressed in the presence of CSF-1-stimulated macrophages from tumor-bearing hosts[95], and CSF-1 suppressed MHC class II expression in the placenta[94]. Other studies have substantiated this immunosuppressive effect of CSF-1 *in vitro*[62, 63], and CSF-1 administration *in vivo* suppressed proliferation of purified splenocytes to T cell mitogens[22].

Apart from targeting MHC class II expression, the immunosuppressive effect of CSF-1 may be dependent on the enzyme indoleamine 2,3-dioxygenase (IDO), which was induced in co-cultures of CSF-1-derived macrophages and T cells[64]. IDO rapidly degrades tryptophan, an amino acid that is essential for T cell proliferation. Several studies have reported inducible expression of membrane-bound CSF-1 on T cells upon activation[34, 72, 88, 102]. Given the inhibitory effects of CSF-1 on T cell proliferation, inducible expression of CSF-1 in T cells may provide a negative feedback mechanism to arrest T cell proliferation via antigen-presenting cell-mediated tryptophan depletion. Despite these studies, the relevance of CSF-1 as an immunosuppressive agent during antigen-specific responses to infectious agents remains to be established. However, CSF-1-mediated effects on T cell function may be relevant to tolerance of the allogeneic fetus during pregnancy. IDO expression in the placenta is critical for maternal T cell tolerance[65], and the most dramatic regulation of CSF-1 *in vivo* occurs during pregnancy where uterine, but not circulating, CSF-1 levels increase 1000 fold[5]. Hence, CSF-1-induced IDO may contribute to the maintenance of tolerance to the developing fetus.

CSF-1 and Chronic Inflammatory Responses

The involvement of macrophages in chronic inflammatory diseases such as rheumatoid arthritis (RA) is well established and the effects of CSF-1 in such diseases have also been studied. In collagen-induced arth-

ritis, a mouse model of RA, administration of CSF-1 exacerbated disease severity whilst an anti-CSF-1 antibody reduced the severity of established arthritis[12]. CSF-1 has also been implicated as a contributor to disease severity in other arthritic models[1, 9]. Evidence exists for CSF-1 involvement in RA itself; CSF-1 levels were elevated in RA patient sera[48] and synovial fluid[27], and synovial fibroblasts from RA patients produce CSF-1[81].

Apart from arthritic diseases, there is an extensive literature on the contribution of CSF-1 to kidney disease. Macrophage accumulation is a predictor of renal outcome in glomerulonephritis and correlates with kidney dysfunction in humans[67], and elevated levels of renal CSF-1 are apparent in glomerulonephritis patients[42]. Other studies have also documented enhanced CSF-1 levels in sera of patients with chronic renal disease[58, 78]. In experimental disease models, there is clear evidence for the involvement of CSF-1 in directing excessive macrophage proliferation and tissue damage. The severity of lupus nephritis in MRL-lpr mice correlated with CSF-1 levels[10], treatment with anti-CSF-1R antibody reduced local macrophage proliferation during experimentally induced renal inflammation[54], and treatment of mice with CSF-1 enhanced LPS-induced glomerular macrophage accumulation and proteinuria[93].

Conclusion

Determination of the exact roles of CSF-1 during immune responses has been difficult because of its essential role in the differentiation, proliferation and normal cellular function of macrophages. Nonetheless, numerous studies have documented effects of CSF-1 on macrophage activation *in vitro* and inflammatory responses *in vivo*. The fact that CSF-1 levels are elevated during responses to infection in mice and humans strongly supports the concept that this factor has key immunoregulatory functions. As with other pro-inflammatory cytokines, CSF-1 exerts protective effects against many infectious agents (with some notable exceptions), but elevated levels of CSF-1 may contribute to pathology in both acute and chronic inflammatory diseases. Hence, specific antagonists of CSF-1 action may have therapeutic potential as a means of preventing pathology associated with excessive macrophage accumulation and activation.

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